has not been identified at the time of this report. Five patients are alive (3–13 mo) while 1 patient died 12 mo. after CBT due to pneumonia.

**Conclusion:** Preliminary analysis suggests that ex vivo EXP in MSC-CB is feasible and may provide rapid engraftment. Accrual to the trial continues.

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### $CD4^+FOXP3^+$ REGULATORY T CELLS MEDIATE SUPPRESSION OF COLONY FORMING ACTIVITY OF LEUKEMIC AS WELL AS PRIMARY MYELOID PROGENITORS

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$CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) are essential intermediaries of peripheral self tolerance. The role of Treg as modulators of the adaptive and innate immune responses has been extensively studied within the last decade. Our laboratory has previously found that in addition to their ability to regulate such responses, Treg cells also possess the capacity to inhibit the colony forming activity of early hematopoietic progenitor cells (PC) of myeloid origin. *In vitro* experiments demonstrated that co-cultures of bone marrow PC with activated Tregs resulted in ~60% decrease of CFU-IL3 levels. Suppression of CFU activity was contact dependent and involved TGF-β1, a Treg cytokine with strong inhibitory properties of early hematopoietic progenitors. To address the regulation by Tregs on bone marrow PC *in vivo*, highly purified (>99.5%) Tregs from B6-$Foxp3^{gfp+}$ mice were co-infused with syngeneic bone marrow into lethally irradiated, B6-wt recipients. Colony forming activity of IL-3 sensitive myeloid progenitors was assessed one week post-BMT and was significantly suppressed ($p<0.02$) in recipients receiving co-infused Treg cells. Based on the ability to suppress differentiation of primary hematopoietic PC populations, we hypothesized that Tregs may also inhibit myeloid leukemia progenitor populations. To test this hypothesis, the myeloid leukemia cell line NFS-60 was examined. The expression of IL-3Ra on these leukemia cells was confirmed by flow cytometry, consistent with their IL-3 dependence for growth and survival. We co-cultured anti-CD3/CD28 activated Treg cells with NFS-60 cells for 2 days and examined their CFU activity in rmIL3 supplemented methylcellulose cultures. Activated Tregs suppressed CFU levels ~ 70%. This inhibition was abolished in transwell cultures indicating the Treg effector activity was contact dependent as observed for primary marrow progenitor cell suppression. Addition of rTGF-β1 inhibited NSF-60 proliferation and experiments involving neutralization of bioactive TGF-β1 in Treg/NFS-60 co-cultures with mAb 1D11 are presently underway. We intend to investigate myeloid progenitor cell activity and tumor cell proliferation in leukemia bearing recipients receiving autologous hematopoietic cell transplants (HCT) together with Treg populations.

# HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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### HIGH RISK OF GRAFT FAILURE IN PATIENTS WITH ANTI-HLA ANTIBODIES UNDERGOING HAPLOIDENTICAL STEM CELL TRANSPLANTATION

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**Background:** Haploidentical stem cell transplantation (HaploSCT) is associated with a graft failure (GF) rate of up to 20%, the causes of which are currently unknown. We hypothesized that anti-HLA antibodies (HLA AB) directed against donor specific antigens are contributing to the development of GF in such patients.

**Methods:** 22 consecutive patients, who received a total of 25 haploidentical transplants, were evaluated prospectively for the presence of HLA AB. The conditioning regimen consisted of fludarabine, melphalan and thiotepa previously described. The presence of antibodies was determined by testing the patients' sera with a panel of fluorescent beads coated with single HLA antigens using a Luminex™ platform; results were interpreted as fluorescence intensity (FI) with FI<500 negative, and 500–1500, 1500–3000 and >3000 considered weak, intermediate and strong, respectively. HLA A, B, C, DRB1, DRB3/4/5, DQB1 and DPB1 were typed by high resolution methods.

**Results:** 6/22 patients experienced GF. Five patients, all females with median age 39 years, had intermediate or strong FI antibody titers, directed against both HLA class I and II antigens, most commonly anti-HLA DRB1. Overall, 4/6 (67%) HaploSCT performed in the in the presence of HLA AB developed GF as compared with 1/19 (5%) patients who did not have antibodies ($P = 0.001$). Moreover, all 4 patients (100%) receiving a HaploSCT in the presence of moderate to high DSA titers failed to engraft (Table). Patients with GF underwent second transplants from the same donors. After the first patient was identified to have GF in the presence of HLA antibodies, treatment with rituximab and plasma exchange was initiated; this treatment appeared to decrease the antibody titers in 2/4 patients enough to allow engraftment (Table). No significant differences were found in the # CD34+ cells infused, # allele mismatched, and # of bone marrow blasts at the time of transplant between the two groups. A review of the pregnancy history (all patients with HLA antibodies were females) showed that the median number of pregnancies was 3 in the GF group (N = 5) as compared with 0 in the control group (N = 6) ($P = 0.1$). In addition, the transfusion history identified a median of 37 units (range 17–65) transfused in the GF group (N = 5) as compared with 15 the control group (range 4–38) (N = 16) ($P = 0.007$).

**Conclusion:** A high rate of graft rejection was observed among patients with donor-derived anti-HLA antibodies receiving a HaploSCT.

**Relationship between HLA antibody titers and engraftment in 5 patients who received a total of 8 haploidentical transplants at MDACC**

| Pt # | AB type | Initial titer | R/PE | Titer after R/PE/ pre first SCT | Engrafted Y/N | Titer after first SCT/ pre second SCT | Engrafted Y/N |
|---|---|---|---|---|---|---|---|
| 1 | A*3201 | N/A | N | + + + | N | NT | Y |
| 2 | A*0211/ | + + +/ | Y | ++/ | N | ++/ | N |
| | B*391301/ | +++/ | | ++/NT/ | | +++/ | |
| | Cw*0702/ | +++/ | | + | | NT/+ | |
| | DRB1*0404 | + | | | | | |
| 3 | DRB1*0701 | ++ | Y | - | Y | N/A | N/A |
| 4 | DRB1*0701/ | ++/ | Y | +++/ | N | -/-/- | Y |
| | DRB4* 0101/ | +++/ | | +++/ | | | |
| | DQB1*0202 | +++ | | +++ | | | |
| 5 | DRB1*0401/ | ++/ | Y | ±/+/ | Y | N/A | N/A |
| | DRB4* 0103/ | ++/ | | +++ | | | |
| | DPB1*0401 | +++ | | | | | |

AB – antibody, R – rituximab, PE – plasma exchange, NT – not tested.

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### FLUDARABINE, MELPHALAN AND THIOTEPA CONDITIONING FOR UNRELATED DONOR CORD BLOOD TRANSPLANTATION

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Cord blood transplantation (CBT) is an established therapy for patients (pts) who lack a matched sibling or unrelated donor. However, the optimal type and intensity of the preparative regimen for such pts is unclear. We studied a conditioning regimen consisting of fludarabine, melphalan and thiotepa in adult pts receiving a CBT.

**Methods:** 37 pts, median age 31 years (2–57), median weight 73kg, with advanced hematologic malignancies (24 with acute leukemia, 13 with lymphoma/CLL) were treated between 8/2003-5/2008.